



## Anthropogenic land-use signals propagate through stream food webs in a California, USA, watershed



Jonathan W. Moore<sup>a,\*</sup>, Timothy D. Lambert<sup>b</sup>, Walter N. Heady<sup>a,2</sup>, Susanna E. Honig<sup>a</sup>, Ann-Marie K. Osterback<sup>a</sup>, Corey C. Phillis<sup>a,1</sup>, Angela L. Quiros<sup>a</sup>, Nicolas A. Retford<sup>a,3</sup>, David B. Herbst<sup>c</sup>

<sup>a</sup> Department of Ecology and Evolutionary Biology, University of California, Santa Cruz, CA 95064, USA

<sup>b</sup> Department of Earth and Planetary Sciences, University of California, Santa Cruz, CA 95064, USA

<sup>c</sup> Sierra Nevada Aquatic Research Laboratory, University of California, Mammoth Lakes, CA 93546, USA

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### ABSTRACT

Human development of watersheds can change aquatic ecosystems via multiple pathways. For instance, human rural development may add nutrients to ecosystems. We used naturally occurring stable isotopes in stream food webs to investigate how land use affects stream ecosystems across a gradient of land development in the San Lorenzo watershed, California. Road density was used as a proxy for land development. We found that streams in watersheds with higher road densities had elevated concentrations of phosphate and nitrate. Furthermore, algal  $\delta^{15}\text{N}$  values increased as a function of nitrate concentration, but saturated at approximately 6‰. This saturating pattern was consistent with a two-source mixing model with anthropogenic and watershed sources, fit using Bayesian model fitting. In sites that had  $>2.6$  km roads  $\text{km}^{-2}$ , anthropogenic sources of N were estimated to represent  $>90\%$  of the N pool. This anthropogenic N signal was propagated to stream consumers: rainbow trout (*Oncorhynchus mykiss*), signal crayfish (*Pacifasticus leniusculus*), and benthic invertebrate  $\delta^{15}\text{N}$  were positively correlated with algal  $\delta^{15}\text{N}$ . Even relatively low density rural human land use may have substantial impacts on nutrient cycling of stream ecosystems.

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### Introduction

Human land-use impacts freshwater ecosystems via multiple pathways, such as through nutrient loading and habitat alteration (Naiman and Turner, 2000). Human activities increase loading of limiting nutrients such as nitrogen (N) and phosphorus (P) (Elser et al., 2007; Holtgrieve et al., 2011), driving cultural eutrophication of aquatic systems (Smith and Schindler, 2009). As hydrologic connectors, streams are recipients of and conduits for these nutrients (Gomi et al., 2002). Thus, streams and their communities can be locally impacted by elevated nutrients but then also propagate nutrients to downstream ecosystems like estuaries and lakes that

may be vulnerable to eutrophication (Smith and Schindler, 2009). Consumers in aquatic systems may also be affected by shifting resource bases associated with land-use changes. Although anthropogenic activities can increase nutrient loading to freshwaters via many pathways (Smith and Schindler, 2009), non-point sources of pollution have proven particularly difficult to quantify, monitor, or regulate (Carpenter et al., 1998).

Stable isotopes are increasingly used to investigate how anthropogenic land-use alters aquatic ecosystems. For example, nitrogen stable isotope ratios ( $\delta^{15}\text{N}$ ) can identify potential sources of nitrogen as well as inform rates of nutrient transformations (Peterson and Fry, 1987; Robinson, 2001). Nitrogen stable isotopes are commonly used to estimate the mixing of anthropogenic sources of nutrients into food webs and ecosystems. Human sewage is generally enriched relative to other watershed sources. This strong contrast of anthropogenic versus background watershed sources provides the opportunity, for example, to locate non-point sources of sewage pollution (Cabana and Rasmussen, 1996; Steffy and Kilham, 2004; Cole et al., 2006; Kaushal et al., 2006; Leavitt et al., 2006). One potentially insightful approach to examining patterns of stable isotopes is to compare mixing models with different

\* Corresponding author. Tel.: +1 778 782 9246.

E-mail address: [jwmoore@sfcu.ca](mailto:jwmoore@sfcu.ca) (J.W. Moore).

<sup>1</sup> Present address: Earth to Ocean Research Group, Biological Sciences, Simon Fraser University, Burnaby, BC V5A 1S6, Canada.

<sup>2</sup> Present address: Moss Landing Marine Laboratories, 8272 Moss Landing Road, Moss Landing, CA 95039, USA.

<sup>3</sup> Present address: Ocean Sciences Department, University of California, Santa Cruz, CA 95064, USA.

potential sources (e.g., Vander Zanden et al., 2005). For example, in a study of streams in an agricultural region, Diebel and Vander Zanden (2009) found that variance in N isotope ratios among stream biota was best explained by inorganic fertilizer application and wetland land cover within the watershed, suggesting the importance of fertilizer-derived nitrate and its removal via denitrification in wetlands. Such approaches may be an important step forward to identify and quantify potential sources of excess nutrients to watersheds (Smith and Schindler, 2009).

The ecological effects of human land use can be illuminated through the study of gradients that span urban to rural developments (McDonnell and Pickett, 1990). In many parts of western North America, people are inhabiting more rural landscapes including the headwaters of watersheds (e.g., Kaushal et al., 2006). Although densities of people may be relatively low in these rural areas, the impacts of such developments on aquatic ecosystems may be large (e.g., Moore et al., 2003; Burtner et al., 2011). Here we used stable isotopes to examine how human land-use alters stream ecosystems in the San Lorenzo watershed, California along a gradient of human land-use intensity. We asked the question: how does human land-use alter patterns of nutrients and isotopes in stream communities?

## Materials and methods

### Study system

We examined 12 sites within the San Lorenzo River watershed (Santa Cruz County, CA, USA) that spanned a gradient of human land-use intensity. Sites were located on the numerous relatively small (first and second order) streams within the watershed and were part of a larger study (D. B. Herbst, unpublished data). From this larger set of candidate sites, sites were chosen to stratify a gradient in human land-use intensity and minimize differences in gradient and stream size. With two exceptions, sites were located on different streams. Elevations in this coastal watershed range from 979 m to sea level where the San Lorenzo River enters the Monterey Bay. The climate is Mediterranean, with 76–153 cm rain yr<sup>-1</sup>. Tributaries drain steep soils of weathering granite, schists, marble, and marine deposits consisting of sandstones, shales and mudstones (Herbert, 2009). Most of the watershed is characterized by dense second growth mixed evergreen redwood forest and sparse rural development.

The San Lorenzo watershed has a history of excess anthropogenic nutrient inputs (Ricker et al., 2001). Of particular concern is that excess nutrient pollution has decreased water quality in the downstream lagoon and nearby ocean beaches; high counts of fecal coliform and other indicators of nutrient pollution have led to public warnings that these areas are unsafe for swimming (Ricker et al., 2001). Sources of elevated nitrate levels in San Lorenzo watershed streams include leaking septic and sewer systems, livestock, and urban runoff (Herbert, 2009). Indeed, as of 2000 there were approximately 14,000 septic systems within the 358 km<sup>2</sup> watershed (Herbert, 2009). It is thought that the steep terrain, seasonally high water tables, episodic stream flows, and limited reduction of nitrates through filtration or denitrification in sandy areas, further contribute to elevated nitrate levels in San Lorenzo River watershed streams (Herbert, 2009).

### Field study

Each site consisted of a riffle-pool sequence ranging from 40 to 60 m in length. Sites encompassed an anthropogenic gradient of the watershed which ranged from locations with little anthropogenic influence to locations with higher levels of anthropogenic

influence such as road crossing and rural development. We used catchment road density (km km<sup>-2</sup>) as an index of human land-use intensity for each site. At each site, we collected primary producers (periphyton), and consumers (benthic invertebrates; rainbow trout, *Oncorhynchus mykiss*; signal crayfish, *Pacifastacus leniusculus*) for stable isotope analyses and water samples to obtain nutrient concentrations. All sampling was conducted in June 20–26, 2009 at near base stream flow conditions.

Primary producer biomass was characterized by algae (periphyton) scraped from cobbles collected from both a region of slow- and fast-water within the sampling site. Previous work has found that water velocity can influence algal stable isotope signatures (Findlay et al., 1999, 2002). These samples were analyzed for stable isotopes separately. However, our analyses ended up focused on nitrogen isotopes and these were similar (see section “Results”). We thus pooled algal samples for analyses. Algae samples were frozen immediately after collection.

At each site benthic invertebrates were collected by a Surber sample (0.5 mm mesh; sampling to a depth of 10 cm) in both a region of fast and slow stream flow. Samples were preserved in 70% ethanol. We note that preservation in ethanol can slightly alter isotope signatures (shifting  $\delta^{13}\text{C}$  approximately 1‰ and  $\delta^{15}\text{N}$  approximately 0.4‰; Venturra and Jeppesen, 2009). We did not adjust for this shift because it is likely relatively consistent within invertebrates that have fairly constrained stoichiometry (as opposed to across taxonomic groups with vastly different stoichiometry). Prior to preparation for stable isotope analysis, invertebrates were sorted and identified to family and functional feeding group (filterer, detritivore, herbivore, or predator) according to Merritt et al. (2008). For each functional feeding group present at a site, one or two samples were selected for stable isotope analysis. While samples were run separately, invertebrates from different functional groups were pooled in subsequent analyses because of the lack of replication within functional groups.

Fish and crayfish were collected by three-pass depletion electrofishing. Block nets at the upper and lower extent of each site prevented movement in or out of the site during surveys. Signal crayfish (*Pacifastacus leniusculus*;  $n=35$ , approximately three per site) and rainbow trout (*O. mykiss*;  $n=65$ , approximately five per site) were selected as focal species as they were the most abundant top aquatic consumers present across the different sampling sites. Orbital carapace length (crayfish) or total fork length (trout) and wet weight (to the nearest 0.1 g) were measured on-site for each sampled organism. Crayfish muscle tissue and rainbow trout caudal fin clips (Hanisch et al., 2010; Heady and Moore, 2013) were collected for stable isotope analysis and immediately frozen.

Algae and benthic invertebrates were oven dried until they reached a constant weight (approximately 48 h at 60 °C), whereas crayfish and trout samples were freeze-dried. To remove <sup>13</sup>C-depleted lipids, all consumer samples were flushed with three cycles of petroleum ether at 1200 psi in a Dionex ASE 200 Accelerated Solvent Extraction System. Algae and crayfish tissue samples were ground into a homogenous powder with an agate mortar and pestle. Larger benthic invertebrates were also ground into a fine powder, whereas multiple individuals of the same species were aggregated into one sample for smaller invertebrates. Trout fin clips were left intact. Samples were weighed into 5 mm × 9 mm (algae, mean ± standard deviation = 4800 ± 31 μg) or 3 mm × 5 mm (benthic invertebrates, 589 ± 53 μg; crayfish muscle, 698 ± 30 μg; fish fins, 673 ± 78 μg) tin capsules (Costech Analytical Technologies).

### Stable isotope analyses

Sample  $\delta^{13}\text{C}$  and  $\delta^{15}\text{N}$  were measured on a Carlo Erba 1108 elemental analyzer coupled to a ThermoFinnigan Delta Plus XP isotope ratio mass spectrometer (University of California, Santa Cruz Stable

Isotope Laboratory). Sample isotope ratios were corrected relative to working standards with C:N ratios similar to the samples. For internationally calibrated in-house standards, analytical precision of  $\delta^{13}\text{C}$  and  $\delta^{15}\text{N}$  was less than 0.2‰.

### Water nutrients

We collected duplicate water samples to measure total nitrate and phosphate concentrations at each site. Unfiltered water samples were collected just under the water surface in acid-rinsed high-density polyethylene (HDPE) bottles and were kept frozen at  $-20^\circ\text{C}$  until analysis. Frozen water samples were thawed, filtered through  $0.7\ \mu\text{m}$  GF/F filters (Whatman), and analyzed for nitrate and phosphate concentrations using a QuikChem 8000 Flow Injection analyzer (Lachat Instruments) at the University of California, Santa Cruz Marine Analytical Labs.

### Isotopes and mixing models

To address our question of how human land-use alters stream N cycling, we used a Bayesian information theoretic approach to estimate models and parameters. Bayesian mixing models allow the use of prior information for parameters and the propagation of uncertainty into estimates of posterior probability distributions of contributions to isotopic mixtures (e.g., Moore and Semmens, 2008; Semmens et al., 2009). Bayesian modeling is based on the premise that the probability of a given combination of parameter values is defined by the likelihood of the data given the model and the prior belief in the parameter set. We used such a mixing model to estimate the proportional contributions of N to algae from two sources, anthropogenic (e.g., leaky septic systems) and background watershed sources. This two-source mixing model was written as:

$$\delta^{15}\text{N}_{\text{mix}} = f_a \times \delta^{15}\text{N}_a + f_b \times \delta^{15}\text{N}_b, \quad (1)$$

where the stable isotope signature of the mixture ( $\delta^{15}\text{N}_{\text{mix}}$ ) was a function of proportional contributions of the two sources, anthropogenic sources ( $f_a$ ) and background watershed sources ( $f_b$ ), and their respective isotope signatures ( $\delta^{15}\text{N}_a$  and  $\delta^{15}\text{N}_b$ ) and where  $f_a + f_b = 1$ . While  $\delta^{15}\text{N}_a$  and  $\delta^{15}\text{N}_b$  were not measured in this study, previous studies have indicated that  $\delta^{15}\text{N}_a$  are generally enriched (prior probability distribution was uniformly distributed between 5‰ and 18‰) and  $\delta^{15}\text{N}_b$  are generally depleted (prior probability distribution uniformly distributed between  $-2\%$  and  $2\%$ ) (e.g., Fogg et al., 1998; Leavitt et al., 2006; Jankowski et al., 2012). Thus, the model estimates the isotope signatures of these two sources, constrained by the prior probability distributions. This model does not incorporate isotopic fractionation on uptake (thereby assuming that no fractionation or that the source isotope signatures have already been adjusted to include fractionation). Given that we also quantified nitrate concentrations concurrently with isotopes, we assumed that variation in nitrate concentrations reflected variation in N available to algae. Thus, we modified the mixing model to directly estimate the amount of N from different sources. This was rewritten as:

$$\delta^{15}\text{N}_{\text{mix}} = \frac{(N_t - N_b)}{N_t} \times \delta^{15}\text{N}_a + \left(\frac{N_b}{N_t}\right) \times \delta^{15}\text{N}_b, \quad (2)$$

where  $N_t$  was the measured concentration of nitrate at the site and  $N_b$  was the unknown amount of that nitrate that was from background watershed sources. The prior probability distribution for  $N_b$  was uniformly distributed between 0 and  $10\ \mu\text{MNO}_3^-$ ; we did not have direct or published measurements of this prior and thus used a wide and uninformative prior. Through assuming that background watershed nitrate was constant but unknown across sites and estimating this parameter, we estimated the contribution of anthropogenic nitrate as  $N_t - N_b$ .

It is important to note that these analyses were focused on the N that was available for algae to uptake and transfer into their stable isotope signatures. Given that algae have relatively fast turnover times, we think that our single measurements of nitrate during low summer baseflow are likely good approximations of N available to algae during this summer baseflow period. Therefore, our period of inference was restricted to this time frame. Furthermore, it is possible that other processes are contributing to observed isotopic patterns which would suggest a potentially different model structure (see section “Discussion”).

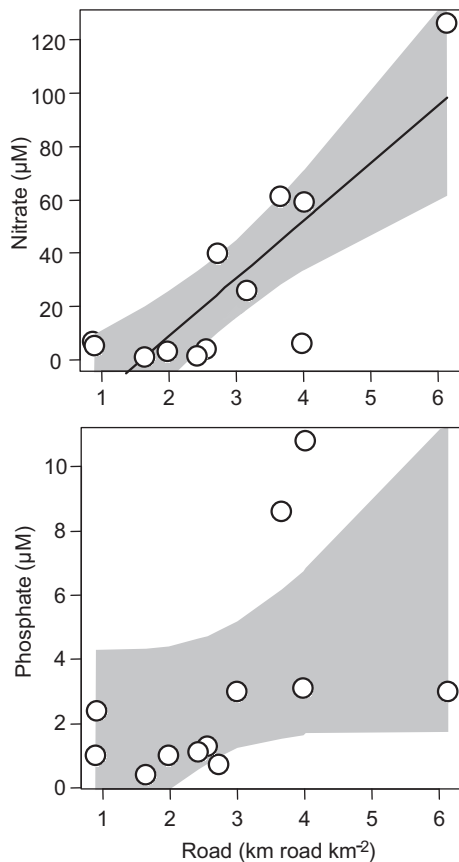
### Bayesian parameter estimation

We estimated the posterior probability distributions of parameters and fit models using a Bayesian approach. Specifically, we estimated posterior probability distributions for  $N_b$ ,  $\delta^{15}\text{N}_a$ , and  $\delta^{15}\text{N}_b$  based on the data and our prior specifications. Bayesian modeling is based on the premise that the probability of a given combination of parameter values is defined by the likelihood of the data given the model and the prior belief in the parameter set. Markov chain Monte Carlo (MCMC) sampling was performed in JAGS in R (Plummer, 2003). In essence, the Markov chains “walk” through the parameter space to map the full probability distributions of the all of the parameters. We used three parallel chains, each with 2,000,000 iterations, with a burn-in phase of 10,000 iterations, retaining every 50th sample. Having multiple chains, a high number of iterations, a burn-in period, and retaining a subset of samples facilitates full exploration of parameter space while avoiding bias from the MCMC process or starting points. Model diagnostics were performed and Fig. 3a, and b were prepared using the coda package in R (Plummer et al., 2006; R Core Team, 2012). All MCMC chains showed visual evidence of convergence, exhibited low levels of auto-correlation, and Gelman and Rubin diagnostics were all at 1, evidence of an effective MCMC procedure. One of the informative outputs of this model fitting is posterior probability distributions of the parameters.

## Results

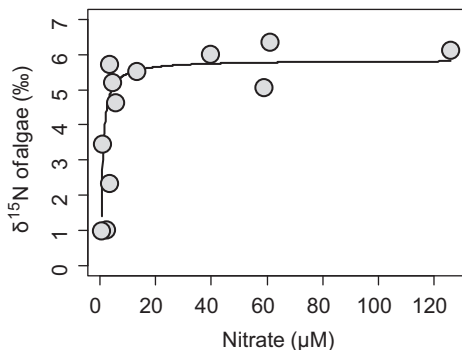
The streams sampled in this survey of the San Lorenzo watershed varied in their land-use intensity. Specifically, the road density of the stream basins ranged from 0.89 to  $6.13\ \text{road km km}^{-2}$ . Sites with higher human land-use generally had higher nutrient concentrations (Fig. 1). Nitrate concentrations were positively related to road density ( $\mu\text{M nitrate} = 21.61 \times \text{road} - 34.17$ ,  $R^2 = 0.69$ ,  $p = 0.0008$ ) and in some sites were extremely elevated (maximum  $126.1\ \mu\text{M}$ ). The relationship between phosphate concentration and road density was not significant although the correlation was positive ( $\mu\text{M phosphate} = 1.05 \times \text{road} + 0.067$ ,  $R^2 = 0.22$ ,  $p = 0.12$ ). The molar N:P of stream water was generally low (median 3.73, mean 11.1, and sd 18.3) and did not covary significantly with road density.

N isotopes of algae showed a strong positive saturating relationship with nitrate concentration (Fig. 2). At sites with low nitrate concentrations, the  $\delta^{15}\text{N}$  of algae was as low as 1‰. Above  $10\ \mu\text{M}$  nitrate, algal  $\delta^{15}\text{N}$  was consistently between 5 and 6.3‰. A two-source mixing model (see section “Materials and Methods”) captured the strongly saturating relationship between  $\delta^{15}\text{N}$  isotopes of algae and nitrate concentration (Fig. 2). Algal  $\delta^{15}\text{N}$  values showed positive but more variable relationships with road density and phosphate concentrations (analyses not shown). These results were focused on the average algae isotope signatures for a site; however we note that the  $\delta^{15}\text{N}$  of slow- and fast-water algae were highly correlated ( $\delta^{15}\text{N}$  of slow-water algae =  $1.04 \times \delta^{15}\text{N}$  of fast-water algae  $- 0.32$ ;  $R^2 = 0.92$ ). C isotopes of algae and consumers were variable and are not discussed further.

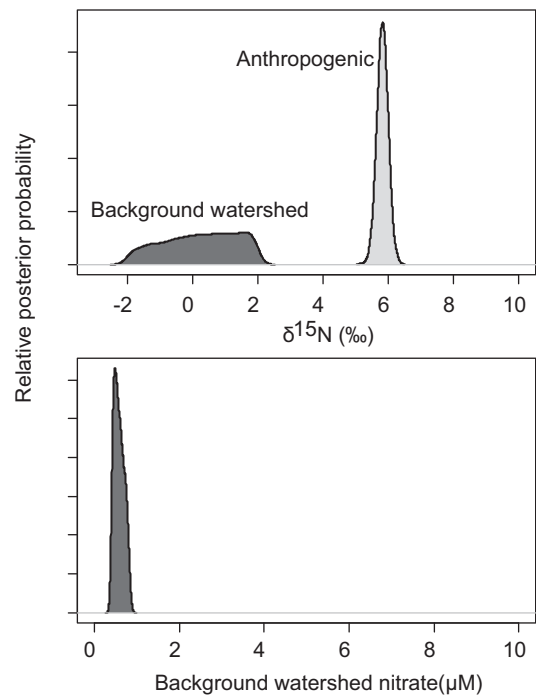


**Fig. 1.** Relationships between road density in stream catchment and dissolved nutrient concentrations. Points represent site-specific average nutrient concentrations. The line shows the best linear fit (significance at  $p < 0.05$ ), and the gray area shows the region contained within the 95% confidence limits.

The parameter distributions from the two-source mixing model revealed information on nitrate dynamics (Fig. 3a,b). The  $\delta^{15}\text{N}$  signatures of the background watershed nitrogen source was estimated to be  $0.32\text{‰}$  with some uncertainty ( $-1.82\text{‰}$  to  $1.93\text{‰}$ ; this and the following represent 95% confidence limits), indicating that the data had little power to distinguish among possible parameter values within the prior distribution. The  $\delta^{15}\text{N}$  value of the anthropogenic nitrate source was estimated to be  $5.84\text{‰}$  (with relatively small confidence limits,  $5.49\text{‰}$  to  $6.18\text{‰}$ ). The model estimated that background watershed inputs of nitrate ( $N_b$ ) contributed little to the total N pool (Fig. 3b;  $0.564\ \mu\text{M}$  with a range of  $0.393\text{--}0.817$ ).



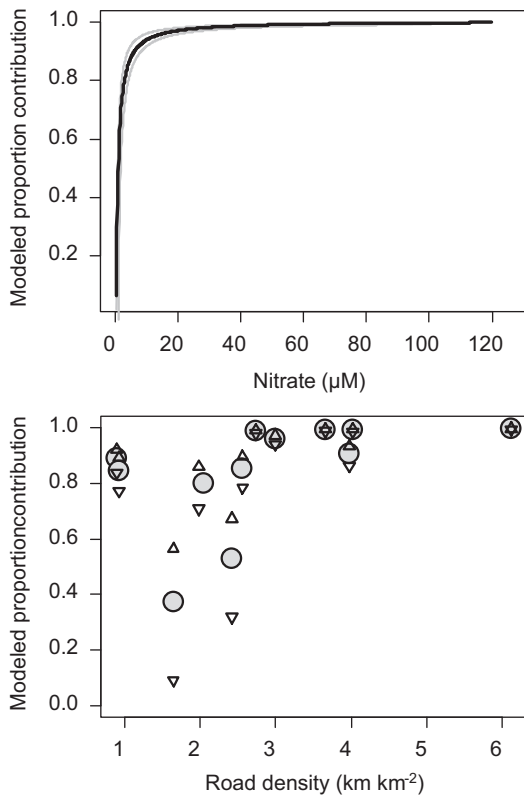
**Fig. 2.** Observed and predicted N isotopes as a function of stream nitrate. Each open point represents the observed average algal N isotope signature and the corresponding site nitrate concentration. Also shown is the line that represents the best model fit for a two-source mixing model.



**Fig. 3.** Parameter estimates for two-source mixing model from Bayesian parameter estimation. Higher posterior densities represent more probable parameter values based on the data. (A) Posterior probability densities for the N isotope value for the background watershed N source and the anthropogenic N source. (B) Posterior probability density of the concentration of nitrate that comes from background watershed sources.

Based on these parameter estimates for background and anthropogenic N, we predicted the relative contributions of background watershed and anthropogenic sources of N to algae across different nitrate concentrations (Fig. 4). At low nitrate concentrations, anthropogenic sources contributed little while background watershed nitrogen contributed the majority of N (Fig. 4a). However, nitrate from anthropogenic sources increased quickly and dramatically, comprising over 90% of the nitrate budget when nitrate  $> 10\ \mu\text{M}$ . Estimates of the proportional contribution for these two sources revealed that N budgets shifted across the gradient of road densities (Fig. 4b). At low road densities ( $< 2.6\ \text{km km}^{-2}$ ), anthropogenic sources contributed variably, from 37% to 89% of N. At higher road densities, anthropogenic sources contributed virtually all of the N ( $> 90\%$ ). Confidence limits surrounding estimates were also wider at lower road densities but quite narrow above  $2.6\ \text{km km}^{-2}$ . Accordingly, the proportional contribution of background watershed N mirrored this pattern, being higher and more variable at lower road densities and smaller at higher road densities.

Anthropogenic enrichment of algal  $\delta^{15}\text{N}$  was propagated up food webs (Fig. 5). Benthic invertebrate  $\delta^{15}\text{N}$  values were positively related to algal  $\delta^{15}\text{N}$  values ( $\delta^{15}\text{N}_{\text{inverts}} = 0.91 \times \delta^{15}\text{N}_{\text{algae}} + 1.67$ ,  $R^2 = 0.783$ ,  $p = 0.0001$ ), as were  $\delta^{15}\text{N}$  values of signal crayfish ( $\delta^{15}\text{N}_{\text{crayfish}} = 0.81 \times \delta^{15}\text{N}_{\text{algae}} + 4.2$ ,  $R^2 = 0.76$ ,  $p = 0.001$ ), and rainbow trout ( $\delta^{15}\text{N}_{\text{trout}} = 0.66 \times \delta^{15}\text{N}_{\text{algae}} + 6.38$ ,  $R^2 = 0.72$ ,  $p = 0.0004$ ). Given that isotopes of consumers generally reflect what they eat, plus discrimination, we can use the slope as a proxy for consumer reliance on algae basal resources. Not surprisingly, these three groups of consumers had different intercepts, suggestive of different trophic levels. For invertebrates and crayfish, the confidence limits of the slopes of these relationships included one, suggesting that the anthropogenic N signal may be propagated up the food web in a one-to-one fashion. The slope for rainbow trout was



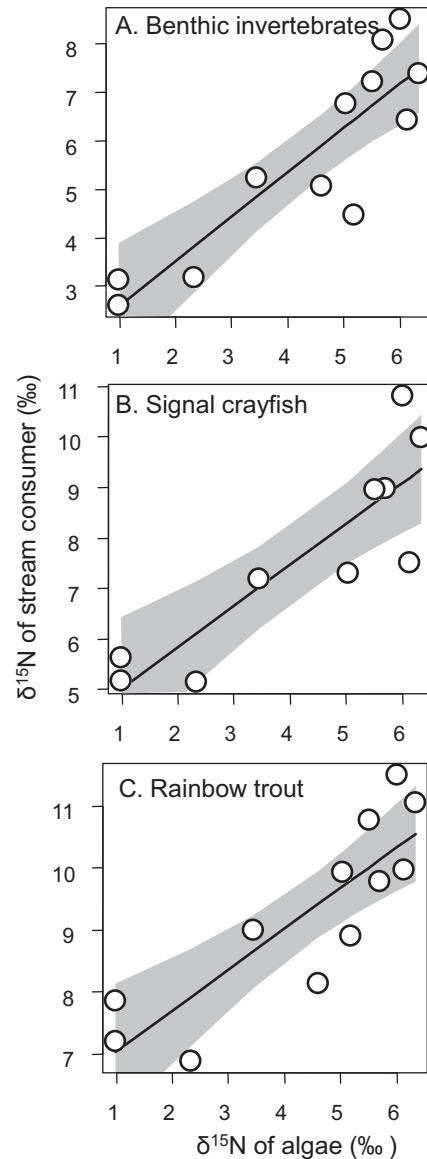
**Fig. 4.** Estimated nitrogen budgets for stream algae, based on the two-source mixing model. (A) The proportional contribution of N from anthropogenic sources, with black line representing best estimate and gray lines illustrating the 95% uncertainty interval. (B) Predicted proportional contribution of anthropogenic N to stream algae as a function of observed road density. Gray points portray the estimate based on the median parameter estimate, and open small triangles illustrate the 95% confidence limits.

significantly less than one, suggesting that these consumers are not fully reliant on local aquatic resources.

## Discussion

Here we quantified naturally occurring stable isotopes in stream food webs across a gradient of anthropogenic influence. Streams in areas with higher human land-use had higher concentrations of dissolved nutrients that can limit productivity (Fig. 1). Intriguingly, nitrogen isotope ratios showed a strongly saturating relationship with nitrate concentrations; at low nitrate concentrations,  $\delta^{15}\text{N}$  values of algae were as low as 1‰, while at nitrate concentrations that were above 10  $\mu\text{M}$ , algae were consistently between 5 and 6.3‰ (Fig. 2). This saturating pattern was consistent with a two-source mixing model that also quantified the contribution of watershed N. Anthropogenic N was estimated to be the dominant source of nitrate; it contributed >90% of nitrate at sites with higher road densities (Fig. 4). These patterns of  $^{15}\text{N}$  enrichment were propagated from algae up to stream consumers such as benthic invertebrates, crayfish, and rainbow trout.

The observed saturating relationship between stream nitrate and algae  $\delta^{15}\text{N}$  is consistent with loading of anthropogenic nitrate (Fig. 2). Indeed, the model explained much of the observed variation in algae  $\delta^{15}\text{N}$  as a function of observed nitrate (Fig. 2). This type of saturating relationship is to be expected from the two-source mixing model that we specified; if we had fit a single source model, then our model estimate would have been a single horizontal line that clearly would not have fit the data suitably (Fig. 2). Our results also speak to the importance of linking measurements of nutrient



**Fig. 5.** N isotopes of stream consumers as a function of algal  $\delta^{15}\text{N}$ . Each point indicates the average isotope signature at each site. The line shows the best linear fit significant at  $p < 0.05$ , and the gray area shows the 95% confidence limits.

concentrations with stable isotopes; stable isotopes yield insight at low nitrate concentrations but get saturated at higher nitrate concentrations.

Our estimates of the anthropogenic  $\delta^{15}\text{N}$  signature provide some insight into the possible mechanisms of anthropogenic N loading. Specifically, Bayesian model fitting estimated the N isotope signatures of the anthropogenic source to be fairly low, at 5.7‰ (Fig. 3a). This value is lower than treated sewage signatures (generally 12–18‰; Hansson et al., 1997; Leavitt et al., 2006; Jankowski et al., 2012), and better matches the N isotopic signature of unprocessed sewage or septic tank effluent (Fogg et al., 1998; Gaston and Suthers, 2004; Hinkle et al., 2008).

It seems likely that the San Lorenzo watershed is awash in N from septic systems during the summer months (Fig. 4). In addition, through making the assumption that the amount of background watershed nitrate was constant across sites; we estimate that background watershed sources of nitrate contribute relatively little to stream loading (Fig. 3b; median estimate of background watershed nitrate was 0.564  $\mu\text{M}$ ). We postulate that anthropogenic loading

leads to elevated nitrate and enriched  $\delta^{15}\text{N}$  of algae. Based on model results, we predict that anthropogenic nitrate dominates in sites with higher human land-use intensity, representing >90% of the nitrate in these areas. By revealing the degree to which anthropogenic sources dominate nitrate dynamics in this watershed, these results build on previous studies in the San Lorenzo river watershed that have suggested that the two primary sources of N are anthropogenic sewage and natural background (e.g., Ricker et al., 1994).

It is important to note that variability in nitrogen stable isotope ratios can be driven by mixing or a variety of transformations via the nitrogen cycle. For instance, N fixation could be an additional source of N, whereby atmospheric  $\text{N}_2$  ( $\delta^{15}\text{N}$  of approximately  $-1\text{‰}$ ) is transformed and added to the ecosystem. N fixation generally occurs when N is limiting, for example when anthropogenic sources are loading nutrients with low N:P ratios (Schindler, 1977). For instance, Jankowski et al. (2012) found that their observed patterns of  $\delta^{15}\text{N}$  in urban lakes was well explained by a mixing model that included mixing of background watershed N, anthropogenic N (from septic/sewage), and N fixation that added enough N to overcome N limitation. We generally observed that the N:P ratio of stream water was low (median 3.73). If fixation is increasing loading of N in the study watershed at a consistent proportion to the anthropogenic N loading pool, N fixation could be contributing substantially to the N budget and observed isotope patterns. However, it is easier to envision N fixation occurring at a rate to reach stoichiometric equilibrium (e.g., Redfield ratio) for lake systems with relatively long residence times (e.g., Patoine et al., 2006; Jankowski et al., 2012); it is less intuitive for stream systems where local nutrient concentrations may be more dependent on upstream processes. Furthermore, denitrification, whereby nitrate is transformed to gaseous N, could be contributing to variation in algal  $\delta^{15}\text{N}$ . Denitrification enriches the residual nitrate pool in  $\delta^{15}\text{N}$  (e.g., Knöller et al., 2011). A large-scale study of stream denitrification found that denitrification rates were higher in nitrate-laden streams in agricultural and urban regions, but that denitrification uptake velocity decreased as a function of nitrate concentration (Mulholland et al., 2008). Perhaps our observed patterns are evidence of thresholds of denitrification. However, given that denitrification removes nitrate and enriches the remaining nitrate pool, this possibility does not explain the observed saturating relationship between nitrate and algal  $^{15}\text{N}$ . Furthermore, it is possible that there are other unaccounted for anthropogenic sources of N such as ammonium fertilizer that have relatively depleted  $\delta^{15}\text{N}$  (Wassenaar et al., 2006). Thus, this study cannot rule out the possibility that other transformations or sources contribute to the observed N budget, but a simple two-source mixing model with watershed and anthropogenic N sources is most parsimonious.

The anthropogenic N signal was propagated to stream consumers. Stream consumers, including rainbow trout, signal crayfish, and benthic invertebrates, all showed a positive and linear relationship with algal  $\delta^{15}\text{N}$  (Fig. 5). If consumers were deriving the majority of their tissue from terrestrial (allochthonous) sources, then the algal  $\delta^{15}\text{N}$  variability would not be related to consumer  $\delta^{15}\text{N}$  variability. The slope of rainbow trout was less than one, however, indicating that these consumers also rely on terrestrial invertebrates with a more constant isotope signature. The observed coherence between stream algal and consumer N isotopes suggests that consumers are deriving much of their tissue from local aquatic resources, highlighting the potential for strong trophic interactions within this stream food web (Moore et al., 2012).

We found that human land-use, primarily in the form of rural developments, altered nitrogen patterns in this coastal California watershed. Our model comparison suggested that the observed

patterns in the  $\delta^{15}\text{N}$  of algae may be driven by inputs of anthropogenic sources. In this watershed, our models estimated that at sites with low nitrate concentrations, the background watershed was an important source of N (Fig. 4). However, at locations with higher nitrate levels that also corresponded to watersheds with higher road densities ( $>2.6\text{ km km}^{-2}$ ), background watershed sources were minimal ( $<10\%$ ). Instead, in these sites, anthropogenic sources contributed to the bulk of the assimilated N. These results, coupled with the fact that this watershed has a high density of residences on septic systems (Ricker et al., 2001), suggest that leaky septic systems from low-density human developments are drastically changing the nutrient dynamics of this coastal watershed. Previous research has also highlighted the problem of excess nutrients in the San Lorenzo watershed and the downstream lagoon (Ricker et al., 2001). The resulting eutrophication continues to degrade this watershed, its ecosystem services such as clean swimming water, and threatens native species such as imperiled anadromous steelhead (*O. mykiss*) that use the lagoon as rearing habitat (Ricker et al., 2001).

This study thus follows the recommendations of McDonnell and Pickett (1990) to study urban to rural gradients to illuminate how land-use change alters ecosystems and communities. The development of watersheds can lead to multiple drivers simultaneously acting to alter aquatic ecosystems. For instance, residential development along streams can add nutrients. Here we found that rural developments had dramatic impacts on nitrate cycling that were propagated up the food web. Different development patterns will likely drive different changes to ecosystem processes (e.g., Moore et al., 2003; Burtner et al., 2011), but these differences are still poorly understood (Alberti, 2005). While human densities in rural developments may be relatively low, if these developments rely on ineffective treatment of wastes, their nutrient enrichment may dramatically change nutrient cycling in aquatic systems.

#### Author contributions

Contributions to this publication include study design (JWM, DBH), field sampling (JWM, AKO, CCP, DBH), sample preparation and lab analyses (TDL, WNH, SEH, AKO, CCP, ALQ, NAR), data analyses (JWM), and writing (JWM and TDL, with contributions and revisions from WNH, SEH, AKO, CCP, and ALQ).

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